COSMETICS

J.W. Wiechers[1], F.J. Groenhof[1], V.A.L. Wortel[1], N.A. Hindle[2], R.M. Miller[1][3]

Efficacy studies using octadecenedioic acid, a new nature-derived ingredient to even Asian skin tone

pd 2002 (5)
P 2-8

Keywords: Octadecenedioic acid, skin toning scalp dandruff, deodorancy, *in-vitro* clinical studies, *in-vivo* clinical studies

Introduction

For centuries, people have turned to nature to obtain beneficial effects of the world that surrounded them and as a consequence oleochemistry, the chemistry of natural oils and fats, has become the backbone of the cosmetic chemistry. But as our knowledge increased, so did our desire for more and more efficacious molecules. As a result, we can no longer find every desired efficacy in nature. Cosmetic scientists have therefore made modifications of existing chemistries to enhance, for instance, the intrinsic activity of the active ingredient originally found in nature or its delivery to the site of action. Whereas such modifications were originally strictly chemical, more recent attempts to enhance the clinical efficacy of cosmetic molecules have been biotechnological. This type of chemistry that uses either isolated enzymes or whole cells as the biocatalysts has certain advantages over traditional chemistry. Biocatalysts have two specific advantages. Like ordinary catalysts, they facilitate a very specific reaction (and thus end product) to be made. But because they originate from biological sources, they catalyse their specific chemical reactions at their physiological temperature. This allows, for instance, the production of thermo-labile molecules.

Normal yeasts rely on glucose and hydrocarbons such as alkanes, fatty acids and triglycerides for their energy consumption. The hydrocarbons are taken up into the microsomes within the cells and oxidised on one end to form a fatty acid and then at the other end to form a dicarboxylic acid, generally known as dioic acid. These dioic acids are excreted from the microsome and taken up in the peroxisome where β-oxidation takes place, yielding acetyl-CoA. This acetyl-CoA is transferred to the mitochondria where it is inserted into the tricarboxylic acid (TCA) cycle, yielding water, carbon dioxide and energy in the form of ATP (Fig. 1a). We identified a natural mutant of a specific yeast that follows the above-described biological pathway in which the peroxisome is inactive. This yeast will start the bioconversion of alkanes, fatty acids and triglycerides but cannot produce beyond the dicarboxylic acid phase (Fig. 1b). Such natural mutants can survive in nature because they can still obtain their energy via the conversion of glucose. When one feeds this natural mutant with only a very small amount of glucose to prevent it from dying and a relatively large amount of oleic acid as the fatty acid, it will convert oleic acid to octadecenedioic acid. This product cannot be made by traditional oxidative chemistry due to the presence of the double bond in the middle of the molecule. Because oleic acid is a natural product with its own distribution of chain lengths, octadecenedioic acid will also, like all other oleochemicals, have its own typical chain length distribution. Octadecenedioic acid consists predominantly of $C_{18:1}$, $C_{18:2}$ and some C_{16} dicarboxylic acids. It is a solid with a melting point of approx. 64-68 °C, typically in the form of flakes of a yellowish-white colour. The chemical has a limited solubility in lipids such as hexane, an even lower aqueous solubility but a reasonable solubility in polar lipids like ethyl acetate and acetone. Adding polar lipids such as propylene glycol and dipropylene glycol can enhance its solubility in aqueous media.

This article describes the cosmetic efficacy studies that were performed with this molecule. Due its structural similarity to azelaic acid, the C_9-dioic acid, that is active in acne and dandruff, we looked at the anti-microbial activities of this molecule in dandruff, deodorancy as well as its skin toning effects. In each of these efficacy fields, we performed *in-vitro* as well as *in-vivo* studies. The former measure the intrinsic activity of the molecule, the second the clinical efficacy of the molecule and therefore also encompasses skin delivery. Therefore, skin delivery will be discussed prior to the various efficacy fields of octadecenedioic acid: anti-dandruff, skin toning, deodorancy and anti-acne.

Skin delivery of octadecenedioic acid

The clinical efficacy of a formulation is the multiplication of the intrinsic ac-

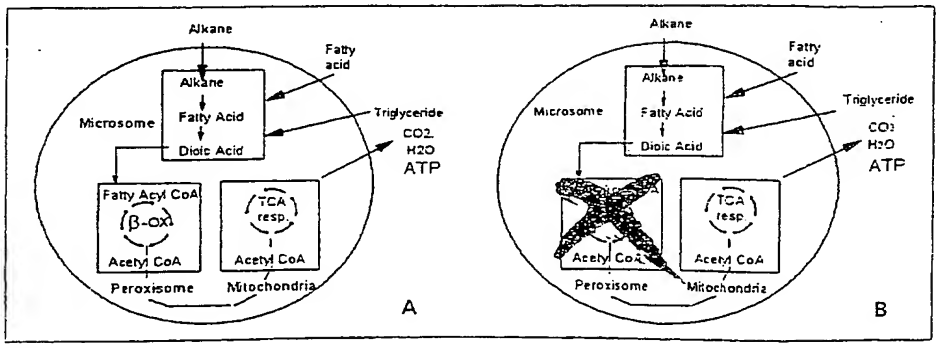

Fig. 1 General biochemical pathways in normal yeasts (A) and specific mutant yeasts (B)

tivity of a molecule and its delivery to the site of action. For skin toning, the active ingredient would have to penetrate as deep as the dermis where the melanocytes are located. For dandruff, it will have to penetrate to the pilosebaceous gland where the *P. ovale* yeast may be located. Finally, for deodorancy, it would have to penetrate to the sweat gland, deep into the dermis, where various organisms convert long-chained fatty acids to the odorous short-chained fatty acids. In short, for all these activities, delivery of octadecenedioic acid to the deeper layers of the skin is required.

Therefore a series of experiments were set up using pig skin as the substrate. The structure of the skin of pigs is very similar to that of humans and therefore pig skin is generally accepted as one of the best models for human skin. Full thickness skin was used, dermatomed to a thickness of 500 µm. This was placed in a Bronaugh flow-through *in-vitro* skin penetration cell system. A continuous stream of receptor fluid (Dulbecco Modified Phosphate Buffered Saline at pH 7.4) underneath the dermis mimics the *in-vivo* bloodstream. Various formulations were applied for 20 hours under non-occlusive conditions. Penetration of ^{14}C-labelled octadecenedioic acid was followed for the duration of the application by measuring the amount of radiolabel in the amount of formulation remaining on the skin at the end of the application period, as well as the amounts in the skin. The latter was subdivided into the superficial amounts retrieved in five tape strips applied consecutively to the application area, and the remainder of the skin, comprising of remainder of stratum corneum and dermis (Fig. 2). In addition, the amount of radioactivity in the receptor fluid, representing transdermal penetration was measured in two-hour fractions.

Results showed a formulation dependency of skin delivery. A first experiment studied the delivery of octadecenedioic acid as well as a structural analogue (azelaic acid) from the same aqueous gel as well as a marketed azelaic acid-containing formulation. This showed that the majority of octadecenedioic acid was delivered transdermally, *i.e. beyond* the site of action (Fig. 3). Only small amounts were delivered dermally. Azelaic acid from the marketed formulation was predominantly dermally delivered as would be anticipated.

Subsequent delivery experiments with o/w emulsions clearly demonstrated that not all o/w-formulations are the same. One particular formulation (for composition, see Table 1) yielded par-

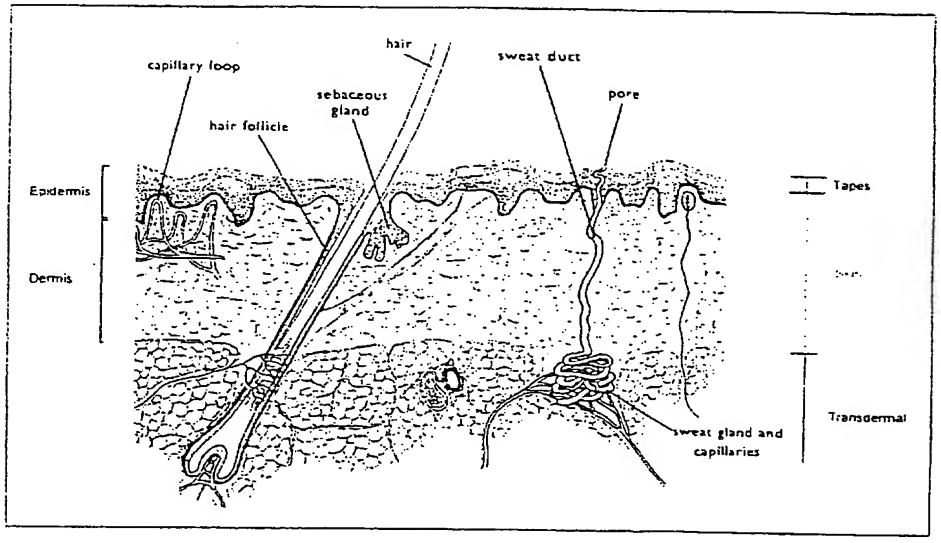

Fig. 2 Schematic overview of skin, indicating which layers are represented by the measurements in tapes, skin and transdermal fractions

Caprylic/Capric Triglyceride	10
Glyceryl Stearate SE	3
Steareth-21	5
Steareth-2	1
Cetyl Alcohol	2
Octadecenedioic Acid	2
Glycerin	3
Benzoic Acid	0.2
2-amino-2-methyl-1-propanol	to pH 5.5
Distilled water	ad 100

Table 1 Composition of the o/w emulsion that demonstrated best dermal delivery of octadecenedioic acid and was subsequently tested in skin toning and deodorancy studies. Concentrations are all given as percentage w/w

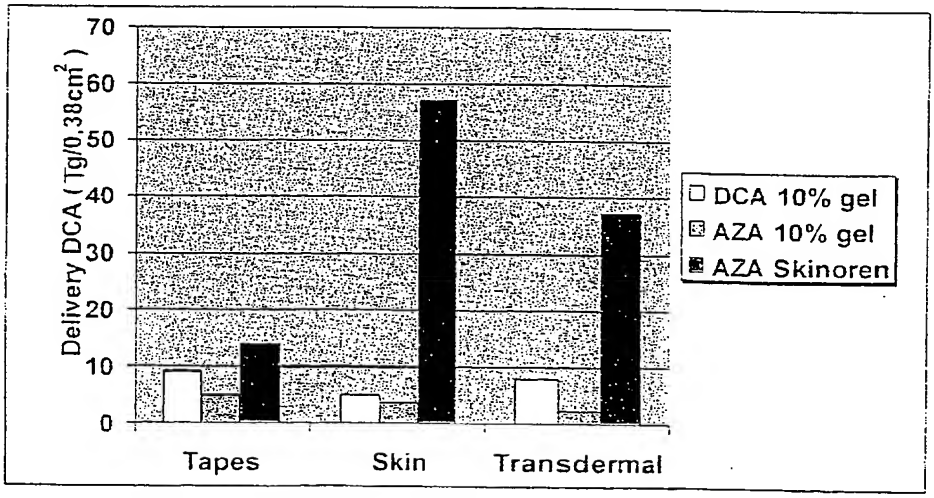

Fig. 3 Dermal and transdermal delivery of octadecenedioic acid and azelaic acid from an aqueous gel and a marketed formulation. Results are expressed as absolute amounts of octadecenedioic or azelaic acid penetrating into the surface area of the skin penetration cell, 0.38 cm²

ticular good dermal delivery, exactly to the location in the skin where octadecenedioic acid should be delivered (Fig. 4). The subsequent clinical studies investigating skin radiance and skin deodorancy were performed using this particular o/w-formulation. These experiments showed that there is a clear formulation-dependency in dermal delivery of octadecenedioic acid. Depending on the type of formulation, penetration to the dermal layers can be achieved. Whether the amount delivered is sufficient to obtain clinical efficacy for octadecenedioic acid in the various applications will be discussed in the following sections.

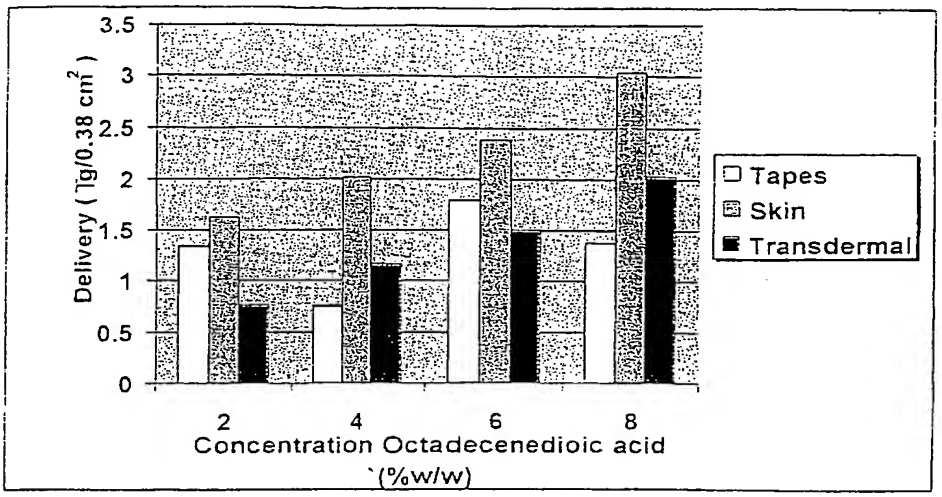

Fig. 4 Dermal and transdermal delivery of octadecenedioic acid incorporated at various concentrations from the o/w formulation as described in Table I. Results are expressed as absolute amounts of octadecenedioic acid penetrating into the surface area of the skin penetration cell, 0.38 cm²

Clinical studies using octadecenedioic acid

Two types of studies exist, *in-vitro* and *in-vivo* studies. The former type measure the intrinsic activity of a molecule to exert a certain effect whereas the latter type combines the intrinsic activity with the capability of the molecule to reach its site of action. Therefore, *in-vivo* efficacy studies or clinical studies are seen as the ultimate evidence of the activity of a molecule. Octadecenedioic acid was tested for its efficacy in anti-dandruff, skin toning and deodorancy. In all cases, *in-vitro* studies, in which other benchmark molecules were also included, preceded the clinical *in-vivo* studies.

Anti-dandruff studies with octadecenedioic acid

In-vitro studies measuring the intrinsic efficacy towards anti-dandruff revealed only a moderate efficacy of octadecenedioic acid against *Malassezia furfur*, the yeast thought to cause or at least to be associated with chronic scalp dandruff. Its anti-fungal efficacy was comparable to that of salicylic acid. Despite this, its clinical efficacy was exceptionally good, suggesting that additional mechanisms such as, for instance, a keratolytic action, may also be involved. A first study, investigating various concentrations on small panels to identify the effective range of octadecenedioic acid, revealed 5 % in Johnson's Baby Shampoo™ (Johnson & Johnson) to be active in reducing the clinical signs of dandruff against placebo (Fig. 5). Subsequent studies using 2 and 5 % on normal-size panels showed significant reductions in dandruff ($p = 0.036$ and $p < 0.001$, respectively). Another study compared the efficacy

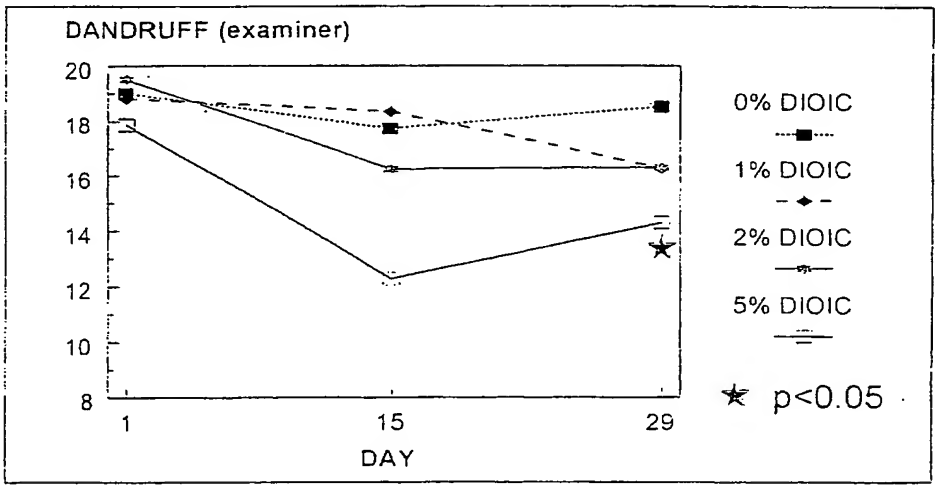

Fig. 5 Antidandruff efficacy of octadecenedioic acid in concentration range-finding studies: Dandruff scores of products containing varying amounts of octadecenedioic acid on very small panels (only 8). Additional studies on full panels gave the significance levels indicated in the text

of 2 % octadecenedioic acid in Johnson's Baby Shampoo with Head & Shoulders™ and found the two shampoos to be similar in their antidandruff performance until the very last evaluation at 4 weeks. All these studies were performed by simply added octadecenedioic acid to Johnson's Baby Shampoo without formulation optimisation, suggesting that with some additional formulation work to enhance the deposition, even better anti-dandruff shampoos can be created with octadecenedioic acid.

Skin toning studies with octadecenedioic acid

In *in-vitro* skin toning studies, the efficacy of octadecenedioic acid was compared to that of, amongst others, hydroquinone, kojic acid and arbutin. In contrast to these molecules, octadecenedioic acid did not inhibit tyrosinase activity. In a clinical study on Indian and Pakistani subjects, the 2 % octadecenedioic acid containing o/w-emulsion as described in Table 1 was applied twice daily for 8 weeks on one forearm whereas as a similar formula-

tion without octadecenedioic acid was applied on the other forearm. Skin colour development was measured on weeks 0, 2, 4 and 8 by means of a Chromameter®, a Mexameter®, an Erythemameter and by visual assessment. At the end of the trial, all instruments indicated significant fading of skin colour ($p < 0.025$), whereas the visual assessment was just not significant ($p = 0.055$), possibly due to the use of an ordinal scale in visual assessment. *Fig. 6* showing the arm of a subject before and after application for 8 weeks of a 2 % octadecenedioic acid formulation, however, illustrates the degree of skin toning that may be anticipated.

Deodorancy studies with octadecenedioic acid

The *in-vitro* studies investigating the anti-microbial activity of octadecenedioic acid and triclosan against 20 bacterial strains normally found on human skin revealed that triclosan was more active on a weight basis than octadecenedioic acid. However, triclosan killed basically everything whereas octadecenedioic acid showed a specific activity towards those micro-organisms that are known to cause the formation of the shorter chain fatty acids that are responsible for the malodour in the armpit. Subsequent concentration range-finding clinical studies on very small panels revealed that octadecenedioic acid was extremely efficacious in reducing the malodour (Fig. 7). A single application already gave a significant reduction that only further improved as the number of applications increases. Because of the demonstration of efficacy on such small panels, larger panels were not evaluated but it is anticipated that concentration at levels of 1 % and possibly below may still be efficacious.

Other clinical studies with octadecenedioic acid

In addition to efficacy trials, extensive safety testing of octadecenedioic acid (to allow VIIA status in Europe) has been performed but results of these investigations will not be discussed here. However, the results of two safety tests will be discussed: the three-application patch test that measures skin irritation on multiple exposure as well as the ocular irritation test.

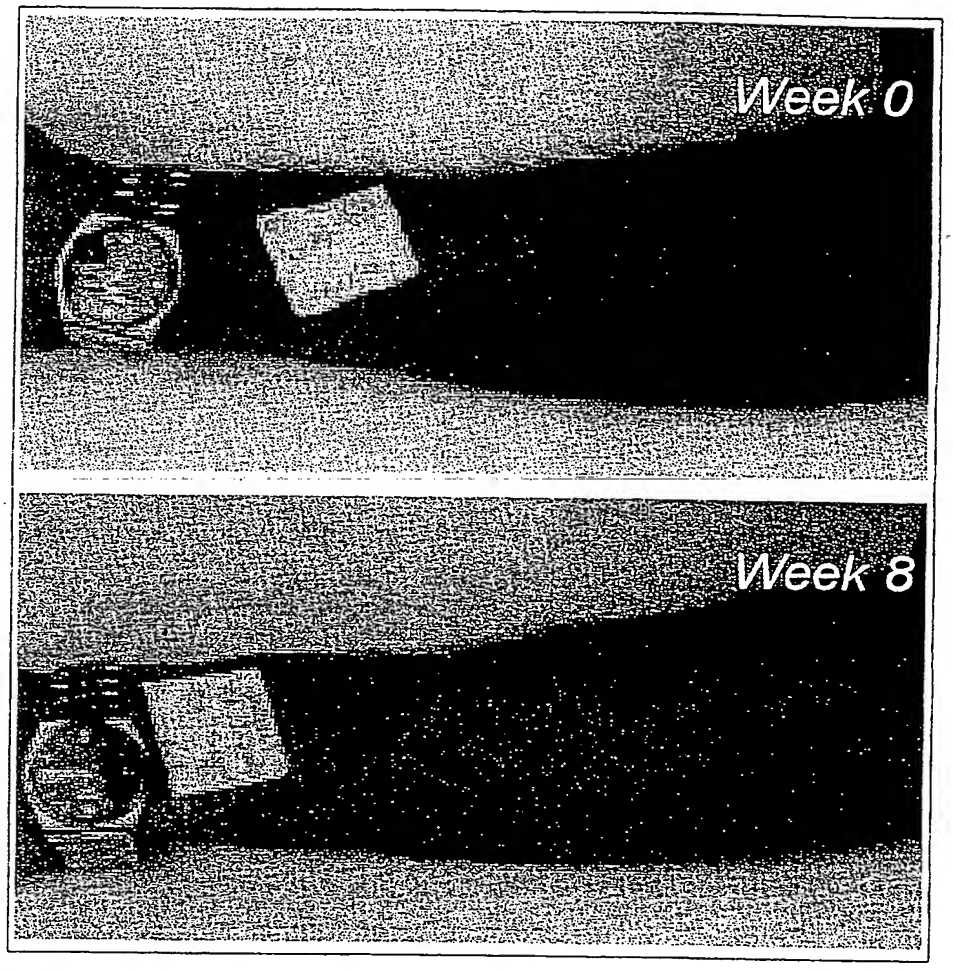

Fig. 6 The arm of a subject before and after eight weeks application of a 2 %-containing octadecenedioic acid containing formulation as given in Table 1

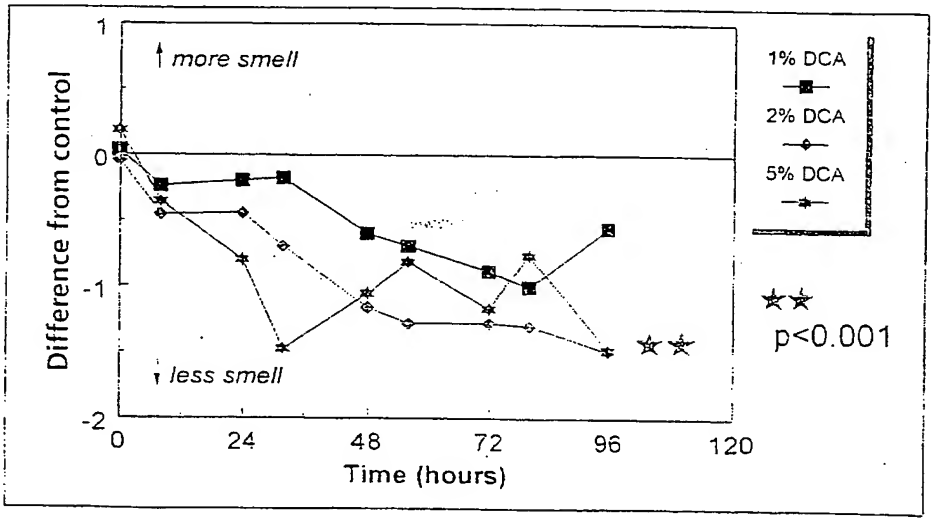

Fig. 7 Deodorancy efficacy data of octadecenedioic acid at various concentrations relative to the placebo product. The general composition of the test formulation is given in Table 1. Note that statistical significance is only indicated at 96 hours for reasons of clarity

Skin irritancy

Our first study investigated the mildness of octadecenedioic acid on human skin. We applied 400 µl of a solution of octadecenedioic acid at 20 % w/w in dipropylene glycol on 1 cm^2 on the upper left arm under occlusion. Dipropylene glycol, 0.3 % sodium lauryl sulphate (SLS) and water were also applied on adjacent sites at the same time. The water and 0.3 % SLS served as positive and negative control for mildness, respectively. Solutions were kept in place for 24 hours after which they were removed. One day after removal, the sites were judged visually on a 4-point scale for erythema and the occurrence of papules and oedema. Thereafter, solutions were re-applied on the same sites and the procedure repeated a second and a third time, according to the schedule in Fig. 8. Positive and negative controls were vastly different in their degree of irritation. Dipropylene glycol was devoid of any irritancy when applied neat on the skin. This revealed octadecenedioic acid at a concentration of 20 % w/w to be as mild as the positive control for mildness, water. This concentration of octadecenedioic acid is probably a lot higher than will ever be incorporated into a cosmetic formulation intended for the cosmetic market, but beautifully demonstrates the lack of irritancy of octadecenedioic acid.

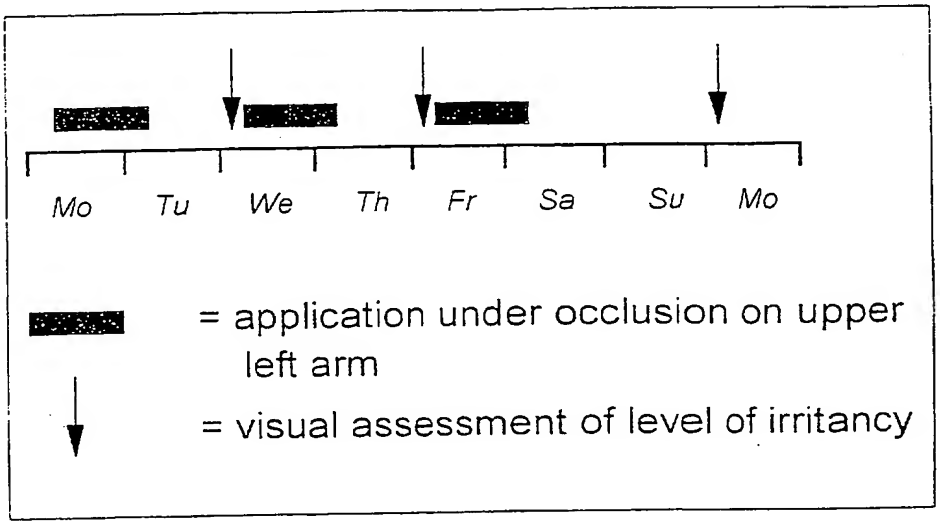

Fig. 8 Schematic overview of the three-application patch test used to investigate the mildness of octadecenedioic acid

Eye irritancy

It is generally known that the human eye is much more susceptible to irritants than human skin. Eye irritancy of octadecenedioic acid is of particular relevance when formulating it into shampoos in order to utilise its demonstrated anti-dandruff properties. Another study was undertaken in which Johnson's Baby Shampoo™ (Johnson & Johnson) containing 0, 1, 2 or 5 % octadecenedioic acid was diluted fivefold with water and instilled into the eyes of human volunteers by an ophthalmologist. A next dose level was only dosed after the previous level had been shown to be without side effects. Lacrimation, bulbar conjunctival irritation and palpebral conjunctival irritation were measured at 0, 0.5, 1, 15 and 60 minutes post-instillation by the ophthalmologist whereas the volunteers were asked for any signs of stinging and burning effects at these time points as well. This study revealed that although octadecenedioic acid in the control product was not as mild as the control product alone, octadecenedioic acid elicited only minimal eye irritation for concentrations up to 5 %, the highest concentration evaluated.

Summary

Octadecenedioic acid is a new cosmetic ingredient that is obtained via a biotechnological route. It was tested for its cosmetic properties in a variety of essays and was found to be effective in reducing the clinical signs of dandruff, evening skin tone as well as reducing body malodour. This was achieved via a systematic approach of *in-vitro* studies investigating the intrinsic activity of the molecule, skin delivery studies, ensuring the best formulation to be tested for clinical efficacy as well as extensive clinical studies using expert evaluation, subject evaluation as well as skin bioengineering methods to quantify the obtained results.

In anti-dandruff studies, octadecenedioic acid was demonstrated to be efficacious against the relevant micro-organisms and to be effective at concentrations of 2 % and above whereas further improvements may be possible via formulation optimisation.

In skin toning studies, an activity was demonstrated at levels of 2 % on Asian skin, but its activity is not via tyrosinase inhibition, allowing for synergistic combinations with most other agents active ingredients that are being used in this application.

In deodorancy studies, octadecenedioic acid was shown to be active against the relevant micro-organisms that cause the typical malodour of the armpit. Moreover, this effect could also be shown in clinical studies at levels of 2 and 5 %, whereas it is anticipated that in larger panels lower levels can also be demonstrated to be efficacious.

Finally, results of skin and ocular irritancy studies reveal that this new ingredient is without irritancy that would prevent the above-described efficacies to be used in the cosmetic market.

Author's addresses:

(1) Johann W. Wiechers,
Frans J. Groenhof, Vincent A.L. Wortel,
Uniqema
P.O. Box 2
2800 AA Gouda
The Netherlands
e-mail: johann.wiechers@uniqema.com

(2) Neil A. Hindle
Uniqema
P.O. Box 90
Wilton Centre
Middlesbrough
Cleveland TS90 8JE
United Kingdom

(1) (3) Richard M. Miller
Miller-Klein Associates
Whitford
Flintshire
United Kindgom